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Bürgmann, Helmut; Frigon, Dominic; Gaze, William; Manaia, Célia; Pruden, Amy; Singer, Andrew C.; Smets, Barth F.; Zhang, Tong

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Water & Sanitation: An Essential Battlefront in the War on Antimicrobial Resistance

Authors: Bürgmann, Helmut; Frigon, Dominic; Gaze, William; Manaia, Célia; Pruden, Amy[1]; Singer, Andrew C.; Smets, Barth; and Zhang, Tong

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Abstract*:* Water and sanitation represents a key battlefront in combating the spread of **antimicrobial resistance (AMR)**. Basic water sanitation infrastructure is an essential first step to protecting public health, thereby limiting the spread of pathogens and the need for antibiotics. AMR presents unique human health risks, meriting new risk assessment frameworks specifically adapted to water and sanitation-borne AMR. There are numerous exposure routes to AMR originating from human waste, each of which must be quantified for its relative risk to human health. **Wastewater treatment plants (WWTPs)** play a vital role in centralized collection and treatment of human sewage, but there are numerous unresolved questions in terms of the microbial ecological processes occurring within and the extent to which they attenuate or amplify AMR. Research is needed to advance understanding of the fate of resistant bacteria and **antibiotic resistance genes (ARGs)** in various waste management systems, depending on the local constraints and intended re-use applications. WHO and national AMR action plans would benefit from a more holistic "One Water" understanding. Here we provide a framework for research, policy, practice, and public engagement aimed at limiting the spread of AMR from water and sanitation in both low-, medium- and high-income countries, alike.

[1] **Corresponding Author:** Department of Civil & Environmental Engineering, 418 Durham Hall, 1145 Perry Street, Virginia Tech, Blacksburg, VA 24061, USA. Email: apruden@vt.edu. Phone: (540) 231-3980.

1. Overview

Antimicrobial resistance (AMR) has been hailed as the 21st century's top global health threat, where, without serious and immediate attention, a return to a pre-antibiotic era is being predicted (WHO, 2014). The inability to treat infectious diseases would result in negative public health consequences and a devastating global economic burden (WHO/Europe, 2017). In recognition of this crisis, the **World Health Organization (WHO)** has developed a **Global Action Plan on AMR (GAP on AMR)**, which was subsequently ratified by the United Nations and is being translated by each member state into national AMR action plans (WHO, 2015). The WHO GAP on AMR calls for the need to optimize the use of antimicrobial medicines in human and animal health and the development of standards and guidance for assessing risks from antimicrobial residues in water and wastewater. Notably, while the GAP on AMR acknowledges the risks and need to understand the role of the release of AMR microorganisms found in farmyards, food, water, and the environment, specific advice on policy is not provided, especially with respect to wastewater and reuse. Two years after the publication of WHO's GAP on AMR, the European Commission published "A European One Health Action Plan against Antimicrobial Resistance (AMR)". In this groundbreaking policy document, the EC called for research to address the knowledge gaps pertaining to AMR in the environment and transmission. Specifically, in Section 3.6, the EC calls on member states to support research into: 1) the release of resistant microorganisms and antimicrobials into the environment and their spread; 2) risk assessment methodologies to protect human and animal health; and 3) the development of technologies for the removal of antimicrobials in wastewater and the environment, with the aim of reducing the spread of AMR. It is the aim of this Perspective to expand on this EC One Health Action Plan, as it is the only high-level policy document to explicitly recognize many of the issues that need to be considered in the 'One Water' concept.

Sanitation, defined as the collection and treatment of human sewage, is a cornerstone of reducing the global burden of infectious disease, including those caused by antibiotic-resistant pathogens. Effective water and sanitation practices therefore must be central to any AMR action plan. Here we provide a framework for understanding the role of water and sanitation as a vital aspect in addressing the current AMR crisis. In particular, we focus on **antibiotics** (herein defined as antibacterial agents that kill bacteria or slow their growth and that are used to combat bacterial infections), **antibiotic-resistant bacteria (ARB)**(defined as bacteria with acquired

resistance) and **antibiotic resistance genes (ARGs)** (which confer increased bacterial survival in the presence of antibiotics), particularly those that are mobilizable.

Wastewater treatment plants (WWTPs) are an essential feature of modern urban infrastructure, employing various physicochemical and microbial ecological processes to biodegrade organic pollutants, remove nitrogen and phosphorus, and reduce pathogen loads before discharging the treated water to the environment. Centralized wastewater management systems collect and treat sewage containing human excrement and other waste streams, effectively mixing any excreted antimicrobials along with human microbiome-associated commensal and pathogenic **ARBs** bearing **ARGs** (Su *et al.*, 2017). WhileWWTP infrastructure has improved and increased over the last century, water sanitation and management systems have not been designed intentionally to manage AMR concerns. Understanding the fate of ARBs and ARGs through the spectrum of water sanitation and hygiene practices becomes particularly acute in the modern movement towards recovery of wastewater residuals for fertilizing and use of treated wastewater for irrigating edible crops as well as augmenting potable water supplies (Pruden, 2014). Here we provide a synthesis of an expert panel discussion that took place at the 4th International **Environmental Dimension of Antimicrobial Resistance (EDAR)** Conference, held in Lansing, Michigan, US, in August 2017, and review relevant literature. We identify the most urgent research needs, while also proposing a path forward in terms of common sense practice and policy that prioritize investment based on the type of waste and likely risk that it carries and considering local socioeconomic, geographic, and other constraints.

2. Modern Wastewater Management Systems: A Conduit or Barrier to the Spread of AMR?

Wastewater management and treatment has been instrumental in reducing disease and death caused by fecal-borne pathogens, such as typhoid and cholera (CDC, 1999, WHO, 2017b). Nevertheless, the degree of sanitation and access to safe drinking water varies widely on a global scale, with 80% of society's wastewater still flowing untreated into terrestrial and aquatic ecosystems (UNESCO, 2017). Hence, the United Nations Sustainable Development Goals #6 "Access to Water and Sanitation for all" and #3 "Ensure Healthy Lives and Promote Well-Being for All at All Ages" should help directly attenuate the spread of AMR. Indeed, appropriate collection, management, and treatment of sewage is an essential step in preventing the spread

of many human diseases, and would simultaneously reduce the need for antibiotics and limit the spread of ARB (WHO/Europe, 2017).

WWTPs receive a full spectrum of human microbiome-associated bacteria, including ARBs and associated ARGs. Also, a substantial portion of antibiotics are excreted by humans in an intact form and they are routinely detected in the influent sewage, with partial removal during biological wastewater treatment (Batt *et al.*, 2007, Zhang & Li, 2011, Rizzo *et al.*, 2013). Sources of antibiotics to sewage include households, hospitals, clinics, and pharmaceutical factories. It isn't clear whether residual antibiotics exert selective pressure on human pathogens and commensal bacteria that pass through the WWTP (Bengtsson-Palme *et al.*, 2017). It is often assumed that the antibiotic concentrations in wastewater are below the **minimum inhibitory concentration (MIC)**, but they still may be above the **minimum selective concentration (MSC)** or minimum **metabolic concentration (MMC)**, driving evolution of AMR (Gullberg *et al.*, 2014, Berendonk *et al.*, 2015). In addition, wastewater may contain other compounds, such as metals and/or biocides, including disinfectants such as quaternary ammonium compounds that have been implicated in ARG selection via co- or cross-resistance (Wales & Davies, 2015, Hegstad et al. 2010).

In WWTPs, the high concentrations of active bacteria and their rigorous mixing in the presence of selective agents might support the evolution of new resistant strains , e.g., through capture of new ARGs by integrons coupled to **horizontal gene transfer (HGT)**. HGT can occur through conjugation, transformation, or transduction, all processes facilitated by high concentrations and close-contact of bacteria that is inherent to biological WW treatment. However, measuring the rates at which HGT actually occurs in WWTPs remains an important knowledge gap. Conceptual and quantitative frameworks that describe HGT dynamics in microbial communities have long been proposed (Smets *et al.*, 1990). They might be adapted, with suitable experimental estimators of HGT rates (e.g., Sørensen *et al.*, 2005) and range (e.g., Musovic *et al.*, 2010), to evaluate the role of HGT in maintaining plasmid-borne ARGs in microbial communities, such as those that might occur in WWTPs. Frequent HGT is likely in WWTPs (von Wintersdorff *et al.*, 2016) due to high loads of antibiotics, ARBs, and ARGs. Already, there are reports of enrichment of certain ARBs, such as carbapenem-resistant bacteria (Hrenovic *et al.*, 2017), and ARGs, such as NDM-1 (Luo *et al.*, 2014), and their influence on impacted watersheds (Pruden *et al.*, 2012, Amos *et al.*, 2014) cannot be ignored. Further, even rare

transfer events occurring in sewage can be important, such as a pathogens acquiring a new ARG.

3. What to Monitor? Determining Suitable Treatment Endpoints

Overview

Effective monitoring schemes for AMR in water treatment systems are urgently needed in order to document baseline levels of resistance and to guide investment of resources towards combating AMR. However, there are numerous options in terms of what to actually monitor. Each option presents its own advantages and disadvantages and, where economically and technologically possible, monitoring multiple endpoints provides the most comprehensive evaluation. Through open collaboration and sharing of scientific information, it is possible to work towards a consensus in identifying simpleyet meaningful, monitoring schemes that are widely accessible to water utilities and **low-and-middle income (LMI)** countries alike and that will aid in global comparison of the effects of wastewater treatment technologies.

Antibiotic Residuals and Selective Agents

Monitoring antibiotic residuals is justified given their potential role in theevolution of AMR. Antibiotics have been associated with selection of resistant strains as well as stimulation of HGT, even below MICs (Shun-Mei *et al.*, 2018). However, in WWTP and other aquatic environments selective effects are often weak, not observed, , or results are conflicting; indicating that causal links between environmental concentrations of antibiotics and selectionfor resistance, may not be straight forward (Gao et al., 2012, Knapp et al., 2008). Therefore, the benefit of intensive monitoring of antibiotics to assess risks of antibiotic resistance development and dissemination in domestic wastewater treatment systems is not clear. Antibiotics typically occur at very low concentrations in wastewater (<1 µg/L) and monitoring requires a high level of expertise and expensive analytical equipment, such as LC MS/MS. Thus monitoring antibiotics might mainly be accessible to large water utilities. Currently, there is no guidance on which antibiotics to target, but a combination of those defined as of “critical importance” by the WHO (WHO, 2017a); such as macrolides, beta lactams and fluoroquinolones, and whether they are readily detected in environmental matrices. With regards to other potentially selective agents, such as metals and biocides, we likewise still lack detailed information on their selective potential in wastewater.

Culture-Based Techniques

Direct monitoring of ARBs is of interest because they represent viable strains with well-known traits that can be subject to phenotypic resistance determination. There are numerous ARBs that could potentially be targeted, both Gram-positive and Gram-negative. These can provide insight into classes of resistance of clinical relevance, which can be monitored either in fecal indicator organisms, in pathogens, or using culture-independent methods. Recently, the WHO has identified **extended-spectrum beta lactamase (ESBL)**- producing *E. coli* as a candidate for global surveillance (Matheu *et al.*, 2017). The intention is that the approach relies upon fecal indicator monitoring as the most widely-accessible method while focusing on a resistance phenotype of utmost clinical concern.

Culture-Independent Techniques

The limitations of fecal bacteria as indicators of true pathogens are well-established (Savichtcheva & Okabe, 2006), as are the limitations of culture-based techniques for representing the actual microbial community composition of complex wastewater ecosystems (Gilbride *et al.*, 2006). Understanding microbial ecological processes is essential in assessing the potential for AMR to evolve and spread (Taylor *et al.*, 2011). For example, ARGs encode the ability to resist antibiotics via various mechanisms, such as efflux pump, target modification, and antibiotic inactivation. Various ARGs have been widely monitored in WWTPs for research purposes, including those encoding resistance genes to beta-lactams, tetracyclines, sulfonamides, quinolones, and glycopeptides (Zhang *et al.*, 2009, Yang *et al.*, 2012). ARGs may be present on the chromosome, but oftentimes are associated with **mobile genetic elements (MGEs)**, such as plasmids, phages, and transposons, which can facilitate HGT.

Several options are faced in determining on which ARGs and/or MGE to focus. ARGs providing resistance to critically-important drugs for human medicine; such as carbapenems, 3rd and 4th generation cephalosporins, fluoroquinolones, and colistins, should be prioritized in line with efforts led by international organizations (WHO, 2017a). These high-relevance genes are often rare in environmental systems. On the one hand, their rarity could be an advantage from a regulatory perspective. On the other hand, their low abundance could reduce the sensitivity of the monitoring approach. As such, more abundant but less clinically relevant genes such as sulfonamide-, tetracycline-, or certain β-lactam-resistance genes may be promising indicators of human contamination. Alternatively, the detection of MGEs known to carry large numbers of

ARGs may provide a more comprehensive and sensitive indication of AMR than individual ARGs. A particularly promising target indicator of anthropogenic ARG "pollution" are class 1 integrons, which hold particularly high clinical relevance (Gillings *et al.*, 2015). Another MGE-targeted approach that could be developed into a monitoring "bioassays" to identify the presence of mobile ARGs is the exogenous plasmid isolation techniques (Smalla et al. 2015) .

Quantitative polymerase chain reaction (qPCR) provides a means of precisely quantifying ARG or MGE targets in water matrices. qPCR has the advantage of being fairly accessible, particularly to larger water treatment utilities. Although classical assays can only count a small number of genes at a time, the use of high-capacity PCR arrays can circumvent this problem (Zhu *et al.*, 2013) as it allows parallel quantification of a multiple ARGs. Quantitative data is crucial to assess effectiveness of mitigation and to relate ARG and MGE distributions to climate or socio-economic conditions. However, scientific knowledge has not yet progressed to the point where there are agreed upon ARG targets to prioritize risks or to recommend admissible values for the abundance of ARGs in an effluent. However, there is movement towards prioritizing ARGs encoding resistance to clinically-important antibiotics as well as their ability to mobilize into pathogens. Correspondingly, relative risk ranking approaches are emerging taking these factors into consideration, along with relative quantities compared to a comparable "background" (Martinez et al., 2015; Manaia, 2017).

Shot-gun metagenomic DNA sequencing approaches are becoming more widely applied for profiling the thousands of "total" ARGs, integrons, and MGEs that may be present in a sample, without *a priori* selection of targets (Yang *et al.*, 2013, Li *et al.*, 2015, Pal *et al.*, 2016). The approach provides unique insight, e.g. into the overlap between the resistomes of different habitats (Munck et al.; 2015). Accurate annotation of ARGs from metagenomic data remains a challenge. However, publically-available ARG databases, such as the Comprehensive Antibiotic Resistance Gene database (CARD) (Jia et al., 2017) and the Structured Antibiotic Resistance Gene (SARG) (Yang et al., 2016) database, are becoming more and more robust, while newer pipelines and tools aid in enhancing ARG identification and data interpretation (Arango-Argoty et al., 2016; Arango-Argoty et al., 2018). "Total ARGs" have been noted to correlate with more traditional indicators of effective wastewater treatment (Yang *et al.*, 2014). Further efforts to refine interpretation of metagenomic datasets and to identify which ARGs/MGEs are of concern and in which context will be valuable. While the metagenomic approach is not widely accessible for routine monitoring efforts because of cost, it has already provided a wealth of information

that should be mined to identify the most useful indicators that can then be evaluated using other methods.

A combination of culture and culture independent analysis is also possible, *E. coli* cultures used for microbial water quality assessment can be subjected to whole genome sequencing, yielding an ARG per *E. coli* metric that can be used with *E. coli* density data to estimate human exposure risk. This approach was used to estimate human exposure in bathing waters in England, with 2,500,000 exposure events to 100 or more *E. coli* borne ARGs estimated to occur in 2016 (Leonard et al., 2018a).

Integrated Approaches

Holistic approaches to monitoring and surveillance should include culture-based and culture-independent methods, along with monitoring of basic physico-chemical conditions and the presence of known chemical drivers of AMR, such as antibiotics, metals and biocides. Such efforts can work towards identifying suitable targets for more unified monitoring in the field. In parallel, or potentially as an alternative, new integrative assays may be worth considering. For example, if the question is whether the treated water has the propensity to propagate resistance, then assays could be developed to assess the extent to which exposure to the water (and its constituent microbiome) can lead to transferred resistance (Klümper *et al.*, 2014). Such approaches would be analogous to the Ames test, or other toxicity assays.

4 Risks Associated with Water and Sanitation sources of AMR

Overview

AMR risk is distinct from traditional pathogen risk assessment because antibiotic resistance does not fit the standard paradigms of dose-response and acute illness. Rather, the primary concern is the spread of resistance and the increasing likelihood of humans and animals acquiring antibiotic resistant infections. Risk may be further subdivided into direct human health risk versus environmental risk of evolution and dissemination of new strains of resistant pathogens containing new ARGs and MGEs. Including consideration of HGT of ARGs amongst commensal and pathogenic bacteria is particularly challenging, but important, as is consideration of potential for latent infection. A more holistic “microbiome” perspective may aid in more comprehensive characterization. Here we evaluate existing knowledge and identify key

research needs with regards to developing a comprehensive framework for risk assessment appropriate for AMR.

Epidemiology

Epidemiology is the study of sources, causes, and control of disease at the population-scale. There are numerous epidemiological methodologies available, including molecular source-tracking of outbreak strains. While scant effort has actually been made to link incidence of AMR infections to water and sanitation exposures (Ashbolt *et al.*, 2013, Huijbers *et al.*, 2015); epidemiological frameworks can provide useful guidance to the development of risk models. The release of ARBs derived from animal and human microbiomes deserves particular attention because these bacteria are also the best candidates for transmitting antibiotic resistance back to humans and animals (Harwood *et al.*, 2014, Manaia, 2017). The mainsources of AMR from WWTPs to the environment are aqueous effluent and solids residuals. Humans can subsequently be exposed either through ingestion, bathing, or inhalation of various media influenced by these sources (Ashbolt *et al.*, 2013, Huijbers *et al.*, 2015). For example, vegetables can be consumed that are irrigated either directly or indirectly with WWTP effluent-influenced water or grown in biosolids-influenced soil (Christou *et al.*, 2017, Zhu *et al.*, 2017), skin could be colonized when swimming in affected surface waters (Leonard *et al.*, 2015) or water ingested, and aerosols could be inhaled by WWTP operators or downwind of biosolids application (Carey *et al.*, 2016, Li *et al.*, 2016).

Risk Definition

Human health risk assessment serves to estimate the probability of illness or death among individual members of a population exposed to a given hazard. More broadly speaking, risk assessment serves to estimate probability and severity of an undesired event. For AMR, risk assessment must take into account and weigh multiple aspects: the current or expected future importance to human and animal health of the antibiotic to which resistance is conferred, the pathogenic potential of the specific bacterial populations carrying the ARG, and the expected ease of dissemination and exposure to affected populations. Further, ecological mechanisms of resistance dissemination, such as HGT, are essential to consider. The potential hazards associated with AMR risk include antibiotics and other selectors (e.g., metals, biocides), ARBs (both pathogenic and non-pathogenic), ARGs, integrons, and various MGEs.

Many if not all ARGs, integrons, and MGEs originated in environmental bacteria and did not evolve *de novo* in clinical pathogens (Poirel *et al.*, 2002, Poirel *et al.*, 2005). As ARGs became associated with MGEs, they were transferred to pathogenic bacteria in a process that might have taken place several times during evolution, leading to today's resistance crisis. In summary, a series of events contribute to the recruitment of ARGs from the environmental resistome into human and animal pathogens and can be broadly classified into three general risk classes: (1) recruitment of ARGs by MGEs over evolutionary time; (2) colonization/invasion of human or animal microbiomes by commensal bacteria carrying mobile AMR at medium timescales; (3) development of an infection by an AMR carrying pathogen at short timescales (Ashbolt *et al.*, 2013). Regarding (1), the initial association between ARGs and MGEs are likely to be rare events, but are prerequisite for spreading ARGs within microbial populations and transfer to other hosts, including pathogens. For example, two comprehensive studies indicated that the vast majority of the soil or WWTP resistome components are not associated with MGEs (Forsberg *et al.*, 2012, Munck *et al.*, 2015). Given these bottlenecks and the specific conditions required for positive selection these evolutionary processes may happen on timescales of years or decades. Regarding (2), the colonization of human and animal microbiomes by mobile ARGs is a result of the exposure to individual human or animal-derived ARB. These bacteria from environmental sources may act as potential donors of ARGs associated with MGEs to human and animal gut microbiomes, even in the absence of active infections (Bengtsson-Palme *et al.*, 2015). In this sense, human and animal microbiomes are "biological reactors" for the emergence, HGT, and proliferation of AMR of clinical relevance (Baquero *et al.*, 2008), in part due to the exposure to strong antibiotic selective pressures (Salyers *et al.*, 2004, Woolhouse *et al.*, 2015). Therefore, the invasion of animal and human microbiomes may lead to rapid dissemination and expansion of the pool of mobile ARGs within a medium timescale (or example weeks to years). Upon acquiring an infection, transfers of ARGs from the host microbiome to the pathogen could be triggered by commencement of antibiotic therapy. This risk is defined as the *chronic exposure risk*, a class of risk not directly managed by current regulations pertaining to WWTP effluents or biosolid residuals (LeBlanc *et al.*, 2009, Jeong *et al.*, 2016). Finally (3), the risk of acquiring an infection caused by a pathogen carrying a mobile AMR gene is operating at the shortest timescale because a single event (infection onset) is necessary. This risk is defined as the *acute exposure risk*.

Exposure Assessment

Exposure assessment identifies the media to which human populations may be exposed and determines the concentrations of the relevant contaminants. Relevant water and sanitation exposures to AMR include: contact with raw sewage (e.g., open defecation areas, operators of sanitation facilities- particularly in locales with inadequate sanitation); aerosols generated during wastewater aeration and treatment (especially relevant to sanitation workers) (Laitinen *et al.*, 1994, van den Broek *et al.*, 2009); skin contact and aerosols generated when irrigating crops or recreational fields with reclaimed water (Carey *et al.*, 2016); ingestion of produce influenced by affected irrigation water or biosolids (Christou *et al.*, 2017); skin contact and particulate inhalation associated with biosolids (Dohmen *et al.*, 2017); swimming/recreation in affected surface waters (Leonard *et al.*, 2015, 2018b, Dorado-García *et al.*, 2017), and drinking water influenced by reclaimed or recycled water (Xi *et al.*, 2009, Garner *et al.*, 2016). It is also important to put such exposure in context with contributions from other sources, such as agriculture, aquaculture, meat products, hospital visits, international travel, and other exposures. In this manner, exposure assessment will help identify and rank likely sources and move towards prioritizing management.

While little work has been done to precisely quantify human exposure; abundances of ARBs, ARGs, and MGEs are fairly well-quantified in sewage, WWTPs, WWTP effluent, and impacted natural waters (Ashbolt et al. 2018). There is also emerging literature on the colonization of the human and animal microbiome (Kozak *et al.*, 2009) with environmental resistance carriers (Bengtsson-Palme *et al.*, 2015). The factors that control and constrain the rates of resistance from various sources to human microbiomes remain largely unknown. Templates for such studies exist, e.g., work on the effect of occupational exposure of workers in slaughterhouses or farms on resistance carriage (Price *et al.*, 2007, Dohmen *et al.*, 2017). One study recently attempted to integrate AMR surveillance, human exposure risk assessment and relative risk of colonization in a cross-sectional study of surfers and non-surfers (Leonard *et al.*, 2018b). This study estimated that >2.5 million exposure events to *bla*CTX-M bearing *E. coli* occured in UK bathing waters in 2015 and that surfers were >4 times more likely to be colonized by ***blaCTX-M*** bearing *E. coli* than the non-surfer control group.

Risk Characterization

Risk assessment associated with “acute” risks posed by AMR pathogens in the environment can be conceptualized within existing **quantitative microbial risk assessment (QMRA)** frameworks as dose response curves exist for exposure *vs*. infection risk (Ashbolt *et al.*, 2013). However, very little is known regarding the short or longer-term health risks posed by exposure to commensal or environmental bacteria carrying mobile ARGs that may be transferred to pathogens on skin or in the human gut. However, a recent systematic review of infection risk associated with colonization by carbapenem resistant *Enterobacteriacea* concluded that 16.5% of colonized individuals developed infections (Tischendorf *et* al., 2016). However, this was a study of adult hospital inpatients where the risk of infection may be greater than in the general population. It is difficult to envisage a risk assessment framework that can disentangle the complexity of very rare gene transfer events of ARGs from environmental bacteria to human or animal pathogens. The factors that lead to such rare events have global consequences but might be unknowable due to complexity, although risk factors for some steps in the chain of events leading to emergence of uncharacterized ARGs in human and animal pathogens may be possible (Ashbolt *et al.*, 2013).

Relative Risk Paradigm as a Proxy

AMR in one form or another is now known to be a ubiquitous feature in microbial communities and ARGs can be found even in entirely pristine or pre-antibiotic era microbes (D’Costa *et al.*, 2011). Further, there is evidence that many resistance determinants that now occur in clinical isolates originated from harmless environmental bacteria (Poirel *et al.*, 2002, Poirel *et al.*, 2005). Thus, it is impossible to expect to achieve “zero” ARBs or ARGs in treated wastewater. At the same time natural “background” ARGs can still present risk. However, until risk models are more developed, a reasonable benchmark is to compare AMR profiles in anthropogenically-influenced environments with those of a relevant natural background. Anthropogenic sources like wastewater have repeatedly been shown to carry much higher loads of ARB and ARG than natural waters (Pruden *et al.*, 2012, Amos *et al.*, 2014). In addition, human impact also tends to be associated with a high degree of mobilized resistance elements. For this reason, establishing background types and numbers of ARGs and MGEs as proxies is an important first step for prioritizing risk and selecting monitoring targets. While it is not necessarily trivial to find a suitable “natural” analogue for wastewater, comparison to natural waters as a reference point

(e.g.,Czekalski *et al.*, 2015) provides an estimate of the contamination potential relative to an accepted harmless environment.

Undoubtedly the individual risks of a particular ARG may vary widely (Bengtsson-Palme *et al.*, 2014, Vikesland *et al.*, 2017) and approaches to rank associated risks based on criteria such as importance to human health, mobility, and novelty have been discussed (Martínez *et al.*, 2015). However, exceptions have been noted in which chromosomally-occurring ARGs of clinical importance are also sometimes highly mobile, as was illustrated by CTX-M progenitors to clinically-important beta-lactamases that were mobilized repeatedly among *Kluyvera* spp. in different parts of the world (Poirel *et al.*, 2002). Ideally, facilitated by next-generation DNA sequencing, "resistome"-based risk criteria could be developed. At the same time, for general regulatory purposes, routine monitoring and for defining initial thresholds for action, simple criteria based on a few common and easily measurable endpoints interpreted against suitable background systems will likely be key. Correspondingly, it would be valuable to determine how simple indicators, such as the class 1 integrase gene *int*I1, more broadly represent resistome-level risk (Gillings *et al.*, 2015).

5. Water Treatment Technologies and Management Practices as Barriers Against the Spread of AMR

Overview

Treatment of wastewater streams is an essential tool for managing AMR risk associated with water and sanitation. The fate of ARBs and ARGs through various wastewater treatment processes has been an extensive area of research in recent years. However, a major challenge is now to synthesize information in a form accessible and informative to water and sanitation engineers. Given that risk-based or other standardized AMR monitoring targets have yet to be identified, studies vary in terms of measures by which they evaluate process performance. Effects of the treated water and sludge on corresponding aquatic and soil-based receiving environments is one approach to determining the effectiveness of wastewater treatment for reducing amplification of downstream AMR markers (Pruden *et al.*, 2012, Amos *et al.*, 2014, Burch *et al.*, 2014, Czekalski *et al.*, 2014). The **Global Water Pathogens Project (GWPP)**

provides an on-line compilation of fecal-based pathogens and includes a chapter that comprehensively summarize the fate of ARBs and ARGs in a range of wastewater treatment systems (Ashbolt *et al.*, 2018). For antibiotic compounds themselves, they may be subject to various transformation or phase-transfer processes, such as sorption, biodegradation, chemical oxidation or photochemical transformation (Zhang & Li, 2011). In this section we provide a brief overview of the range of various treatment options available and their *pros* and *cons* for AMR management, with particular attention towards economics and accessibility given local constraints. Movement towards resource recovery and water reuse also place special consideration on wastewater treatment in the context of AMR and the level of treatment required.

Biological Treatment

Aerobic biological treatments include various adaptations of activated sludge, such as enhanced nutrient removal, as well as biofilm-based processes, such as trickling filters. . We cannot here provide a full review on the fate of AMR in WWTPs, but a general takeaway from the literature is that the situation appears complicated and biological treatment does not uniformly reduce total ARGs (Yang *et al.*, 2013).

Extensive monitoring of WWTPs in Hong Kong using metagenomics indicated that 78 ARG subtypes persisted through the biological wastewater and sludge treatment process. A high removal efficiency of 99.82% for total ARGs in wastewater suggested that sewage treatment process is effective in reducing ARGs (Yang *et al.*, 2014). Microbial ecological processes that may be at play during typical biological treatment include persistence (i.e., pass-through from influent to effluent), attenuation and die-off due to non-native environment and active populations of bacterial predators, selection (i.e., enrichment) due to ability of resistant strains to withstand stress, and HGT of ARGs to the resident WWTP microbial community. While continued research is of value to sort out the role of this tangled ecological web and the ultimate reduction of AMR risk in various contexts, it seems wise to present additional treatment barriers when possible, particularly when the water in intended for reuse.

Biosolids Treatment and Reuse

Aerobic biological treatment generally results in two products: treated water and residual sludge. The sludge is a consequence of the high growth rate microbes employed that actively convert organic carbon to biomass. This biomass is rich in nutrients and thus is commonly used as a “biosolid” fertilizer, particularly in the United States, United Kingdom, Germany, and southern

Europe. However, the risk carried by land-application of biosolids is widely debated and in some countries; such as Switzerland, Sweden, and other Nordic countries, they are largely incinerated and converted to energy. In terms of AMR, mass balance analysis reveals that approximately 90% of the ARGs leave the WWTP in the form of biosolids, while only 10% leaves though the aqueous effluent (Munir *et al.*, 2011, Yang *et al.*, 2014). This suggests that biosolids warrant special attention. Anaerobic digestion is generally the norm for biosolids. Several studies have suggested that thermophilic anaerobic digestion, operated between 48^0 C and 55^0 C, is more efficient for removing ARGs (Diehl & LaPara, 2010, Ma *et al.*, 2011, Tian *et al.*, 2016), but thisis not always the case (Zhang *et al.*, 2015). Anaerobic treatment also appears to be better for removal of ARGs than anaerobic treatment (Diehl & LaPara, 2010, Ma *et al.*, 2011), which may be a function of selecting for different bacteria and corresponding suites of ARGs and also because anaerobes are more prone to lose their ARGs due to tighter metabolic energy budgets (Rysz & Alvarez, 2004). Overall, the complexity of biological treatment of biosolids still presents many unknowns. Additional precautions may be warranted, particularly when used as soil amendment for edible crops, such as extended wait times before harvest.

Advanced Treatment Options

Advanced treatment may be advisable in some circumstances, particularly where there is increased potential for human exposure, but comes at a cost. **Advanced oxidation processes (AOPs)**; such as various combinations of ozone, UV, and hydrogen peroxide, employ hydroxyl radicals to aggressively attack organic matter, including antibiotics and other pharmaceuticals (Klavarioti *et al.*, 2009, Homem & Santos, 2011), reducing overall downstream selection pressure (Liu *et al.*, 2017). In addtion AOP also destroy pathogens. AOPs are particularly growing in popularity when the water is intended for reuse, including potable reuse (Schimmoller *et al.*, 2015). While AOPs appear promising for destruction of ARGs and minimizing AMR risk (Dodd, 2012, Liu *et al.*, 2017), this is not widely proven and research is needed to demonstrate that benefits persist into receiving environments and that they are not ultimately a selective pressure for resistant strains. For example, partly due to bacterial regrowth downstream from ozonation, resistance determinants may not be efficiently removed (Czekalski *et al.*, 2016) and ARBs may even be differentially selected (Alexander *et al.*, 2016).

Membrane treatments can range in size cut-offs, where even microfiltration can enhance DNA removal to a considerable extent (Riquelme *et al.*, 2013). **Reverse osmosis (RO)** has become the norm for potable reuse in the U.S. state of California, as well as the Middle East and other

parts of the world, and arguably is the strongest barrier available, essentially only allowing water molecules to pass through. Unfortunately, in addition to extreme cost requirements, RO also produces significant volumes of undesirable brine water, disposal of which is not insignificant. The resulting water is nearly equivalent to distilled water and thus can greatly overshoot the water quality needed for the intended purpose (Lee *et al.*, 2012). Combining powdered activated carbon (PAC) absorption of antibiotics and other micropollutants followed by ultrafiltration, which is less costly and energy intensive than RO, appears to be particularly promising for reducing toxicity and selection pressure towards AMR (Margot *et al.*, 2013), but does produce sludge that would need to be managed.

Disinfection, such as chlorination, is also commonly applied to treated wastewater On one hand, it is a known entity that presents tremendous benefit for eliminating pathogens in water systems. On the other hand, the effectiveness of chlorine for AMR control remains unclear. A handful of studies have suggested that selection of some ARBs is favored by chlorine (Murray *et al.*, 1984, Shrivastava *et al.*, 2004, Xi *et al.*, 2009, Shi *et al.*, 2013), but this has not been widely established and benefits have also been cited (Dodd, 2012).

Low Tech Treatment

In LMICs and regions with decentralized wastewater systems treatment is often achieved using simple wetlands and oxidation ponds. These systems are more sustainable based on economic and infrastructure constraints but provide less complete treatment than a traditional activated sludge WWTP. Few studies of the fate of AMR in such systems have been carried out (Anderson *et al.*, 2013), indicating the need for research to establish effectiveness of treatment technologies appropriate to LMICs (Ashbolt *et al.*, 2018).

Treatment Focused on "Hot Spot" Sources

Alarming levels of several antibiotics have been reported in surface water affected by pharmaceutical manufacturing discharge, as high as several tens of mg/L (Larsson, 2014), and are a serious concern as a "hot spot" for AMR evolution. The release of antibiotics from manufacturing to public sewage streams and ultimately to the environment must be monitored and prevented at all costs, while hospitals may present an additional point-source of concern worthy of scrutiny. Hospital wastewater generally carries higher loads of ARBs of greatest clinical concern (Varela *et al.*, 2013, Music *et al.*, 2017, Timraz *et al.*, 2017). In addition, the range of antibiotics found in hospital wastewater can differ greatly from that of municipal sewage

owing to the unique suite of antibiotics only available through intravenous injections (Szekeres *et al.*, 2017). Where treatment is employed, biological processes are not uncommon (Liu *et al.*, 2017, Yi *et al.*, 2017), yet are not ideal because the treatment process is not optimized for the removal of the pharmaceuticals, nor are they engineered to reduce or remove ARGs from the wastewater. More extreme on-site treatment of "hot spot" wastestreams aimed at destruction, mineralization or complete retention of antibiotics through AOPs (Hollender *et al.*, 2009), sorptive processes (Li & Zhang, 2010, Bonvin *et al.*, 2016), and/or membranes is preferable (Li *et al.*, 2004, Kovalova *et al.*, 2012, Margot *et al.*, 2013).

6. Towards Global Action in Combatting Water and Sanitation Routes of AMR Dissemination

Need for Improved Global Sanitation

The absolute first step to combat environmental dissemination routes of AMR is to ensure that at least basic sanitation needs are met. Livestock management provides an illustrative example, where poor herd management results in elevated disease pressure necessitating greater antimicrobial use. Parallels to humans might be made in so much as disease transmission is more likely in areas where clean **water, sanitation and hygiene (WaSH)** is not rigorously maintained. The challenge from AMR is maximized in highly populated areas that have poor adherence to WaSH principles and have unregulated use of antimicrobials, characteristics that are found in many of the most populous nations, e.g., India. In 2015, 12% of the world population practiced open defecation, with the highest rates (> 50%) found in Eritrea, Niger, Chad, South Sudan, Benin and Togo (WHO/UNICEF, 2015). Rates of open defecation have been decreasing significantly worldwide, with India and Pakistan, for example, seeing declines from 66% to 40% and 40% to 12%, respectively. Open defecation is commonly found in countries where WaSH is poorly supported and antibiotics are poorly controlled, a combination that further exacerbates the transmission of AMR infections. This is exemplified in India where more and more powerful broad-spectrum antibiotics are being used in the clinic with diminishing effectiveness resulting in an alarming mortality rate of 58,000 newborns per year due to AMR (Laxminarayan *et al.*, 2013). Such a focus on treating infection, rather than prevention, is a false economy. Filice et al. (Filice *et al.*, 2010) showed that the cost of treating patients infected with **methicillin-resistant *Staphylococcus aureus* (MRSA)** was more than

double that of patients infected with **methicillin-susceptible *Staphylococcus aureus* (MSSA)** ($34,657, compared with $15,923) (Filice *et al.*, 2010). In a larger multi-country study, O'Neill showed that improvements in water quality and sanitation across the four middle-income countries of Brazil, Indonesia, India and Nigeria can lead to a reduction in antimicrobial use by approximately 300 million cases per year (O'Neill, 2016). Critical to recognize is that the spread of AMR does not recognize borders, with the local actions putting the global population at risk as a function of global travel, contamination of food products, and even atmospheric dissemination (Mazar *et al.*, 2016). Thus, such "hot-spots" of epidemiological transmission require urgent attention to avoid global dissemination (Graham *et al.*, 2014).

More developed countries are not fully protected against the spread of waterborne ARB, as they grapple with aging infrastructure along with demand for more innovative and sustainable water treatment technologies. In the U.S., the century old water infrastructure; which is plagued by leaky sewers and overloaded WWTPs, most recently earned a grade of "D+" on the American Society of Civil Engineers Infrastructure Report Card based on the physical condition and needed investments for improvement (ASCE, 2017). ASCE further estimated that 271 billion dollars is required to meet sanitation needs in the U.S. over the next 25 years. Poor infrastructure in the form of leaking sewage pipes and their co-location with leaking potable water is a sure way to degrade public health and exacerbate AMR (Karkey *et al.*, 2016). The urgent need to invest in water infrastructure also presents the opportunity to do so in a more sustainable manner, such as advancing water reuse. Since 2008 in the U.S., funding for recycled water distribution has increased by 21% (ASCE, 2017). Water recycling brings obvious concerns for public health, including closing the loop for AMR to disseminate directly from sewage to drinking water (Pruden *et al.*, 2013). However, these are generally being addressed proactively through requirements for aggressive water treatments and multiple barriers (EPA, 2012, National Research Council, 2012), including AOPs and membranes, which are likely to also pose benefits as barriers to AMR, though further research is needed to confirm this is the case. Still, it is critical to recognize that water reuse is happening all around us, whether we recognize it or not, and may be better off as a planned, rather than *de facto* endeavor. For example, in London, a substantial portion of the drinking water supply is derived from the River Thames, which itself receives wastewater from over 2.5 million people by the time it reaches London (Singer *et al.*, 2014). A discussion with wider society about conserving and recycling water is needed given the scarcity of freshwater. The question is not "if" we will need to close the water loop, but "when" and "how". With this in mind, focused scientific effort is

needed to inform policy and support major societal decisions such as how to best prioritize and address mounting public health issues, where to invest in water infrastructure, how to effectively protect source waters, and what level of treatment is acceptable technologically, economically, and socially. In addition to addressing concerns about AMR, improved and upgraded water treatment systems can address other concerns, such as hormones, pharmaceuticals, personal care products, nanoparticles, and microplastics, along with the myriad of other pollutants occurring in municipal wastewater.

Water & Sanitation in the Broader Context: Interconnections with Livestock, Agriculture and Industry

Effort is needed to quantify relative contributions of various anthropogenic and natural sources of AMR in a water sanitation context, including: industry, landfills, livestock manure, agriculture, transportation, stormwater, greywater and mining (Singer *et al.*, 2016). A geospatial and temporal inventory of antimicrobials, metals and biocides released into the environment would be beneficial towards understanding the relative role that each might play in driving AMR in the environment. This can help guide focused mechanistic research and mitigation efforts. MGEs must also be characterised for their roles in maintaining and disseminating existing and novel ARGs, cassettes, plasmids and bacteria (Pruden *et al.*, 2006, Singer *et al.*, 2016). While most antibiotic resistance assays are determined under idealized conditions, ideally consideration to chemical mixtures along with co- and cross-selective pressures and in realistic environmental matrices is needed (Bengtsson-Palme & Larsson, 2016, Le Page *et al.*, 2017, Xu *et al.*, 2017).

Agriculture accounts for 70 percent of all water withdrawn from aquifers, streams and lakes (FAO, 2011). In many large rivers, only 5 percent of former water volumes remain in-stream, with some rivers having insufficient water to reach the sea year-round (Rutkowski *et al.*, 2007). This rapid increase in irrigation agriculture over the past 50 years represents a major driver for water reuse (e.g., grey and blackwater (Drechsel *et al.*, 2008, National Research Council, 2012). Wastewater residuals, such as biosolids, are also commonly applied as soil amendments (Qadir *et al.*, 2010). The exposure of grazing animals to pasture amended with reclaimed wastewater and biosolids represents another poorly explored pathway between humans, the environment and animals (Eamens & Waldron, 2008). Differences in the carriage of AMR genes between human and animals could also be used to develop treatment endpoint criteria. For example, a Dutch metaanalysis found differences in the frequency of specific mobile AMR genes carried by ESBL *E. coli* from human origin (including wastewater) and farm animal

origins (Dorado-García *et al.*, 2017). Similarly, a European-wide study found mobile fluoroquinolone-resistant genes carried by *Salmonella* to be present essentially in human and poultry, but not in other farm animals (Veldman *et al.*, 2011). Finally, a Czech study found the vast majority of *aac(6')-Ib-cr* (mobile resistance to fluoroquinolones) carrying *E. coli* isolates in samples of human (specifically clinical) origins, but not from poultry origins (Röderova *et al.*, 2017). The identification of specific marker genes would likely need to be done on a regional basis, but the human and farm animal resistance surveillance data available in several regions could be used to provide necessary information to make adequate selections of indicator AMR genes.

Opportunities for Policy

WaSH has been at the center of many of the Millennium Development Goals (UN, 2017) and remains core to the Sustainable Development Goals (UN, 2015). Although sustainable access to safe drinking water and basic sanitation is a major goal proposed by the WHO, in 2015 2.4 billion people were still using unimproved sanitation facilities and 946 million people were still practicing open defecation. The importance of proper sanitation for effective control of AMR was recently recognized by the WHO in their Global Action Plan (WHO, 2015). Effective policy to limit AMR dissemination through appropriate handling of WaSH continues to go unrecognized, in LMI and High Income Countries, alike. While AMR is not the only problem in the world today, it is one that affects everyone in one form or another. Thus, it is reasonable to recommend investments based on the best available science and that are economically in harmony with other benefits to other systems providing fundamental needs, such as food, water, and healthy ecosystems. Continued advancement of the science, such as identifying meaningful monitoring markers and developing appropriate risk models, will help guide the most fruitful focus of such investments. For example, should the focus be on what goes down the drain in the first place, or on targeted treatment of "hot spot" point sources, like agricultural application, hospitals, and pharmaceutical manufacturing? Further, should the bulk effort be focused on water, which is more widely disseminated, or sludge, which contains higher loads of resistance determinants? Also, simply requiring the provision of basic information to consumers, such as where their drugs are coming from and under what conditions they were produced, can help support an informed populace in voting with their purchasing power if the goods do not comply with their environmental ethics and principles. In terms of water quality regulations, systematic documentation of the occurrence of various ARBs and ARGs is needed, which could help

advance the simple fecal-source tracking frameworks that have long been recognized to be fraught with shortcomings.

While acute risks of human pathogens, including resistant strains, is, to some extent, considered by regulations on treated wastewater and biosolids application (LeBlanc *et al.*, 2009, Jeong *et al.*, 2016); broader risks on the ecology and evolution of new strains, and risks of colonization and delayed infection are largely uncharacterized and unaddressed. It is especially difficult to pinpoint risks associated with releases of various ARB, ARG, and selective targets without agreed upon and standardized assays. Still, given the enormous potential harm of an escalating antibiotic resistance crisis, it may be prudent to follow a precautionary principle, and assess where and how improvements to the current situations are feasible. Within the water sector, possible interventions include: i) strengthening existing barriers to AMR dissemination ii) introducing new barriers and iii) reducing selective pressure for AMR **(Figure 1)**.

Communication, Education, and Engagement

Scientific research plays an essential role in advancing the state of the knowledge, while scientists and scientific advisory panels can offer advice at local, global, and national levels on how to most effectively harness water supply and sanitation practices as a means to limit the spread of AMR. However, concerted action requiresbuy-in from all stakeholders, including pharmaceutical manufacturers, hospitals and medical practitioners, water and sanitation engineers, government, and the public.

Ultimately the public sways and prioritizes investment of public funds towards the myriad of problems in today's world. Thus, keeping the public informed about the problem of AMR is essential. While a growing number of people experience the devastation of resistant infections, the problem remains often narrowly viewed as one of hospital "infection control". Given the essential nature of antibiotics to public health, incorporation of units on antibiotic resistance in the public school curriculum, with emphasis on microbial ecological and evolutionary processes as well as environmental routes of dissemination, is warranted. In today's globally-interconnected society, working with the media is also an essential avenue to an informed public. However, the AMR problem is complex, and involves numerous unknowns and uncertainties, making it difficult to accurately boil down concepts into a simple message for a lay-audience. Poorly managed communication can be disastrous if it induces fear without pointing out clear courses of action, eventually fueling growing mistrust in science. Itis

important to recognize that risk perceptions of stakeholders are often different from those of the scientists and that the majority of people rely on their own unique “lay theory” to understand and make sense of the world (Science for Environment Policy, 2014). Ultimately, improved public knowledge will be a driving force towards mitigating the spread of AMR. Pointing out comprehensive benefits of related action, such as improving sanitation and conditions in developing countries, may help encourage and mobilize the public.

Education and training of professionals involved in water quality policies and management as well as experts in environmental conservation and protection can do much to translate science into practice. Engaging with various scientific communities, is a necessary step towards improved water and sanitation practices considerate of AMR risk. Also human health care and veterinary practices would benefit from more integrated knowledge of the ecology and evolution of AMR, moving beyond the confinement of the clinic or the farm and spanning people, animals, and the environment.

Synergies with One Health-One Water

Managing the chronic risk of exposure of humans and animals to AMR by limiting the release of antibiotics, ARBs, ARGs, and MGEs from human wastewater streams to the environment is worthy of immediate scientific and policy action. Ultimately, the reality is that our food, sanitation, and potable water systems are intimately connected and interdependent systems that provide a foundation for public health. This is the essence of the “One Water”/”One Health” paradigm (One Health, 2017, One Water, 2017), which can provide a guiding principle for recognizing these interconnections and steering action. Success will require building and fortifying new channels of communication among multiple stakeholders and between stakeholders and science and ensuring that they are engaged and understand their role in the larger AMR challenge (Singer *et al.*, 2016). It is only through a holistic vision of the many interconnected threads that a robust and sustainable policy can be formulated.

As scientific guidance builds and strengthens, there are several avenues by which a precautionary principle rationally applies (Foster *et al.*, 2000, Maynard, 2002). AMR concerns can be factored into already planned WWTP upgrades or water reuse plans. Policy pertaining to sludge disposal and land-application of biosolids can consider effects on long-term persistence of ARGs and MGEs and their potential for dissemination in the air, into surface- or ground-water, and into food crops and animals (Singer *et al.*, 2016). Ideally, a precautionary approach

can be incorporated with minimal additional investment as synergistic opportunities with other initiatives and benefits are identified. The tension between rapid action and need for evidence-based policy will remain a significant challenge for many years to come. Solutions to the AMR challenge in the environment will require technological, medical, behavioral and societal change. As a global society, it is critical to continue to seek to identify and implement such changes within a holistic framework, incorporating the perspectives of policymakers, regulators, scientists, industry and society. It is only through this inclusive holistic strategy, at the national and international level, that gains can be maximized and unintended consequences minimized. It will take a concerted effort by ALL nations to make this progress and ensure continued benefits of antibiotics to public health for future generations.

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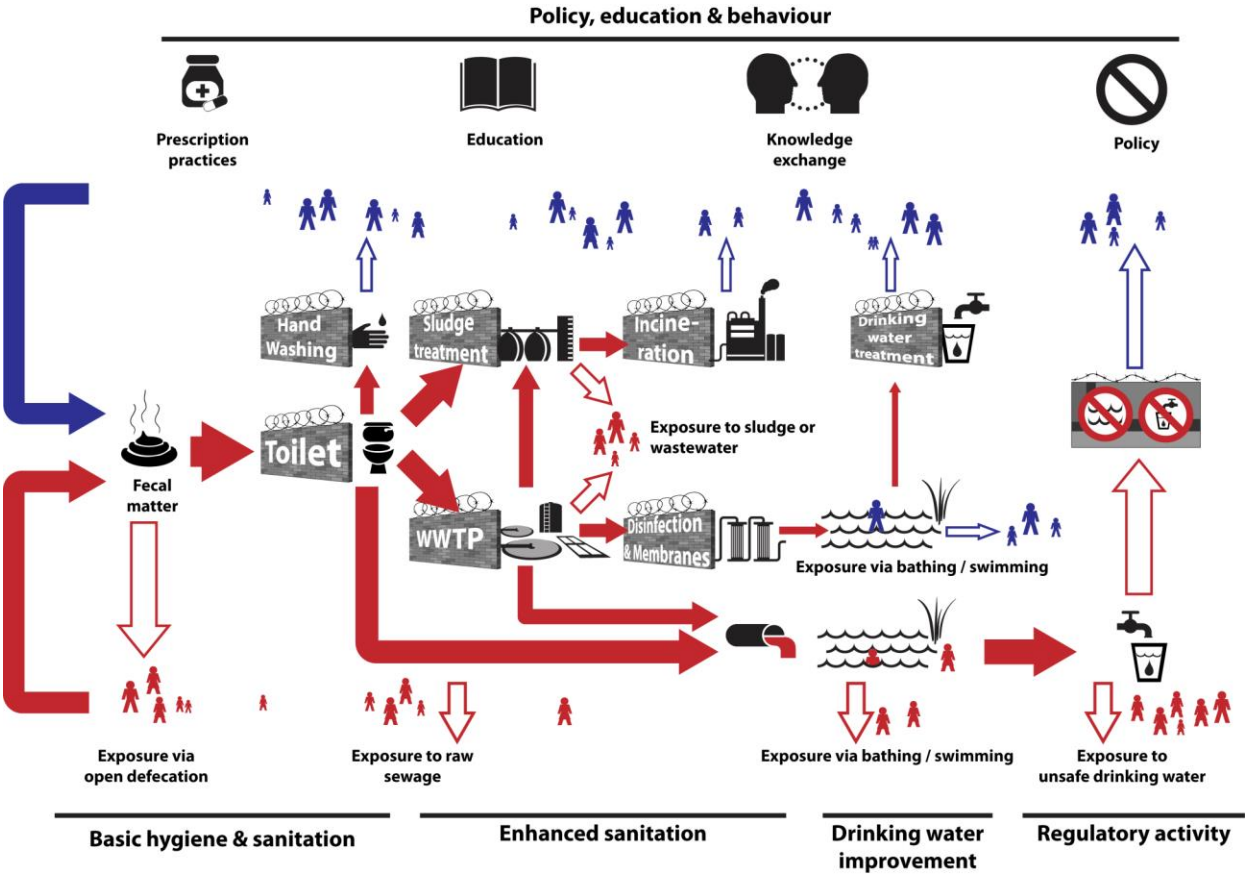

Promising barriers to environmental dissemination of antimicrobial resistance along the water and sanitation continuum and ultimately to human exposure.